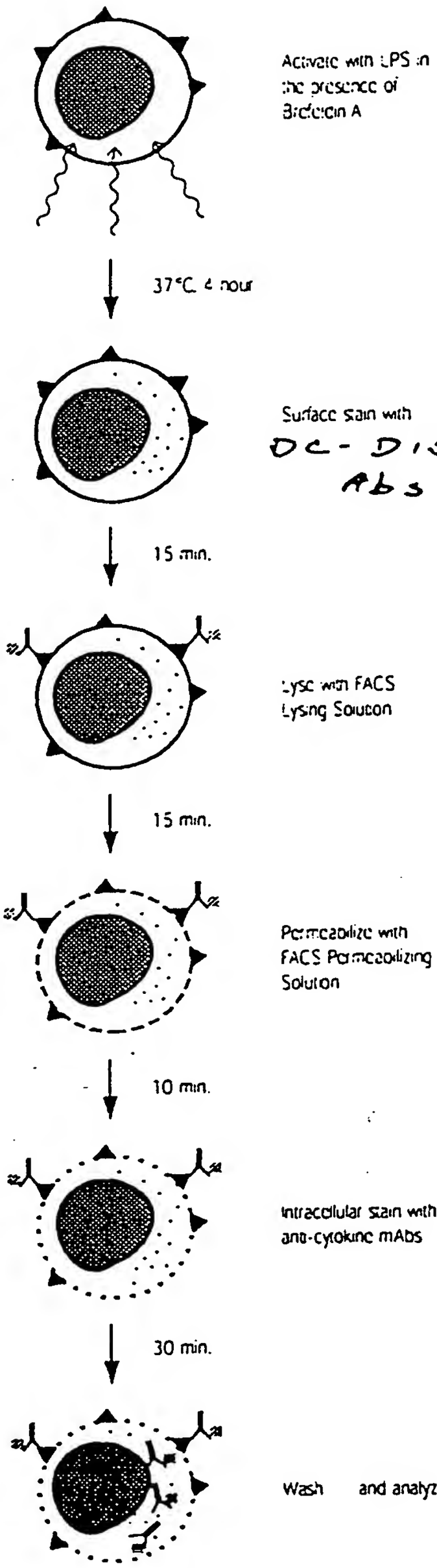
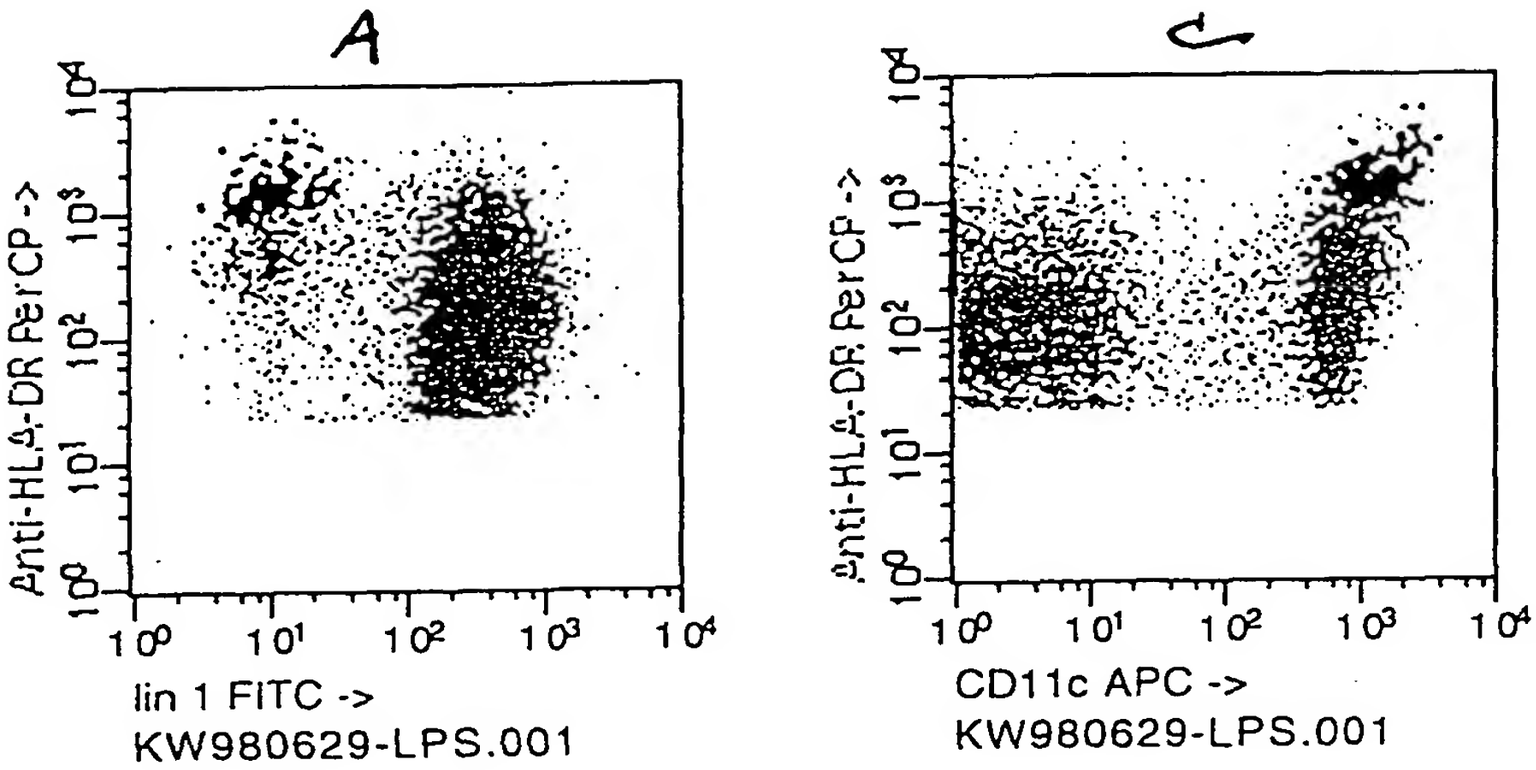

Activate with LPS in
the presence of
Brefeldin A
37°C, 4 hour
Surface stain with
DC-DISTINGUISHING
Abs
15 min.
Lyse with FACS
Lysing Solution
15 min.
Permeabilize with
FACS Permeabilizing
Solution
10 min.
Intracellular stain with
anti-cytokine mAbs
30 min.
Wash and analyze

Fig. 1

Identification of CD11c+ and CD123+ (CD11c-) peripheral blood DCs

-LPS activated-



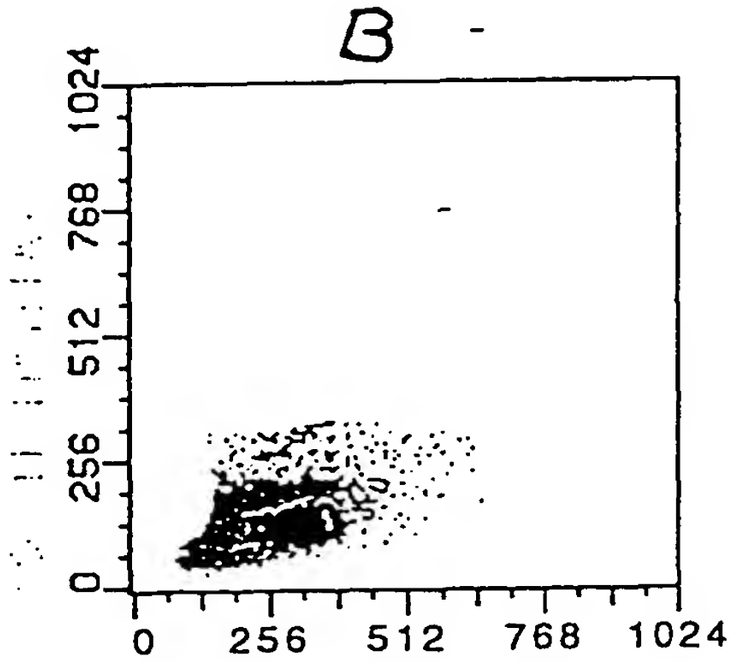


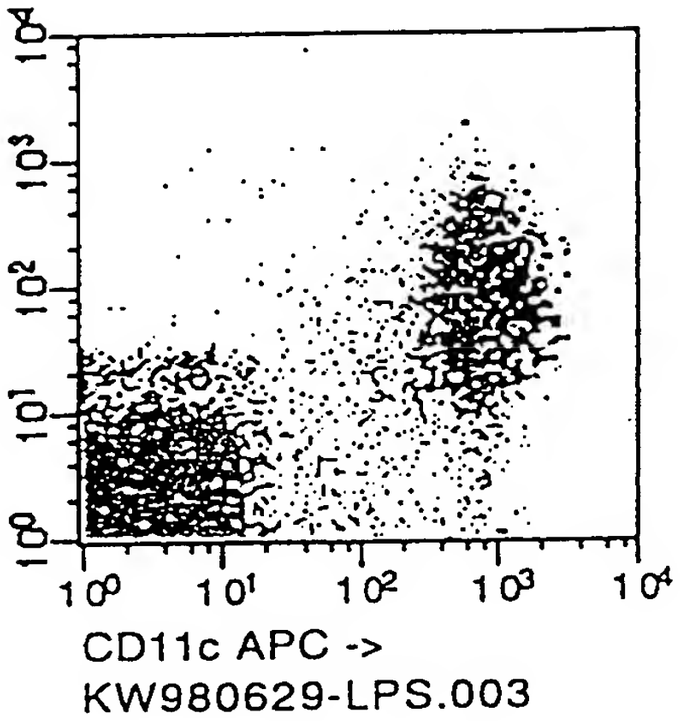
Fig 2

Detection of cytokines in CD11c+ and CD123+ (CD11c-) peripheral blood DCs

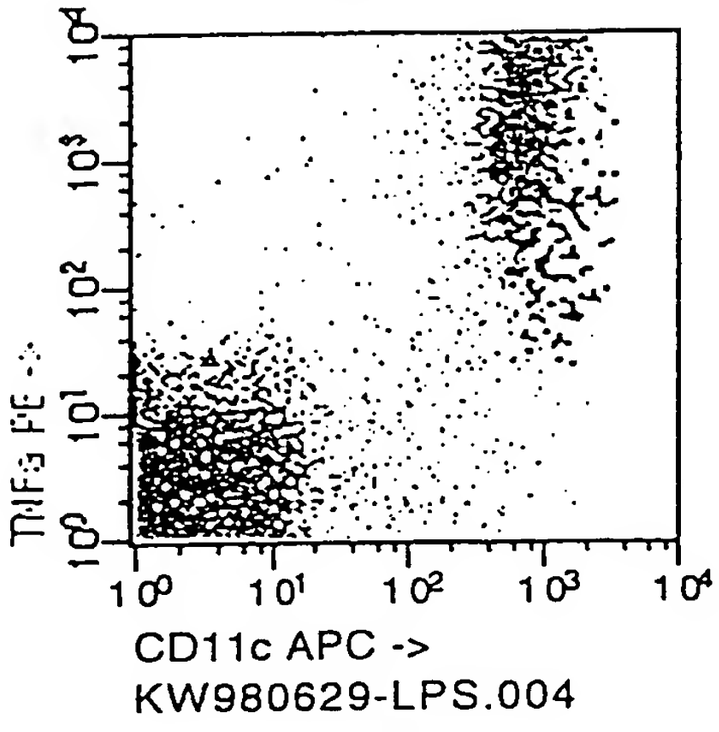
-LPS activated-

D

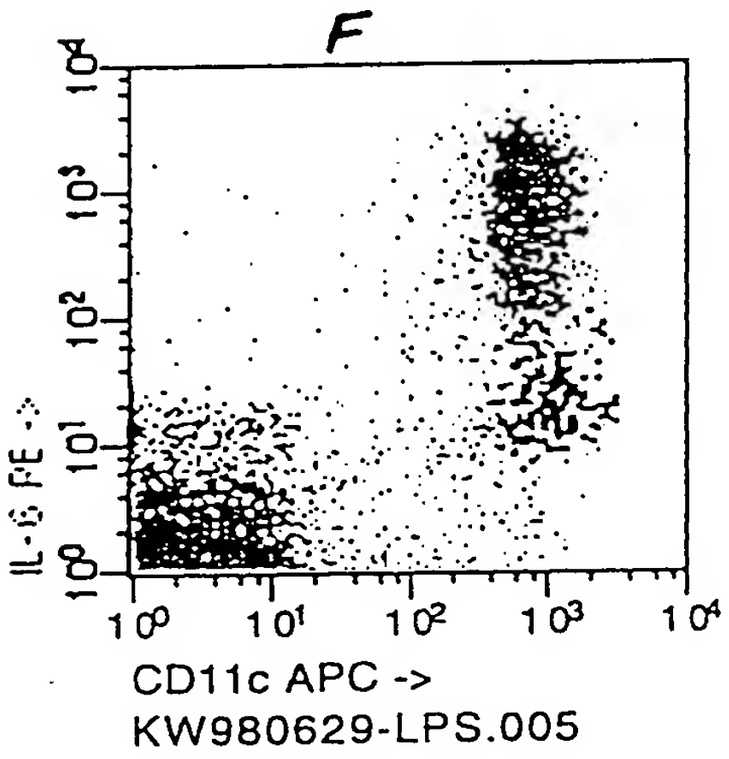
y-axis = IL-1RA PE



E



F



G

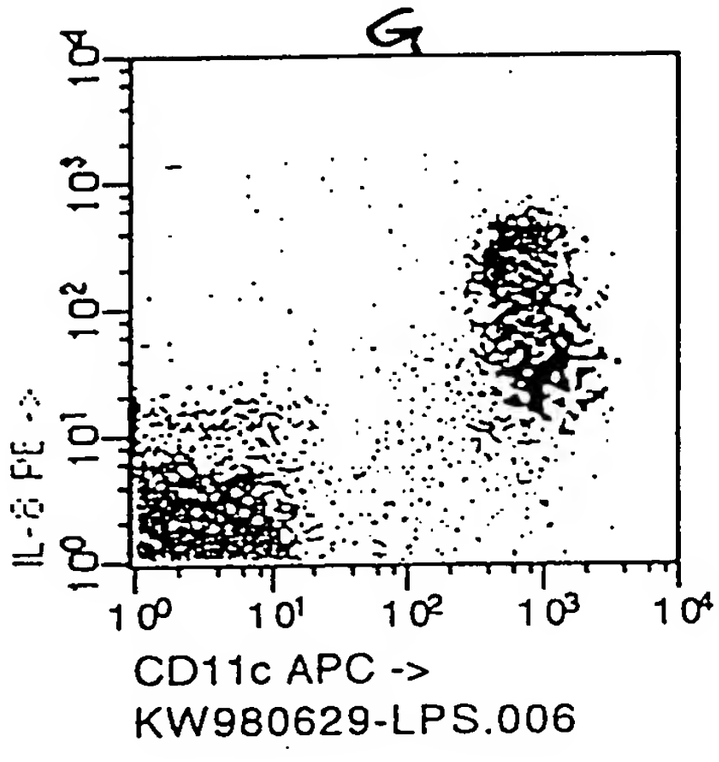


Fig 2 (CONTINUED)

Detection of cytokines in CD11c+ and CD123+ (CD11c-) peripheral blood DCs

-LPS activated-

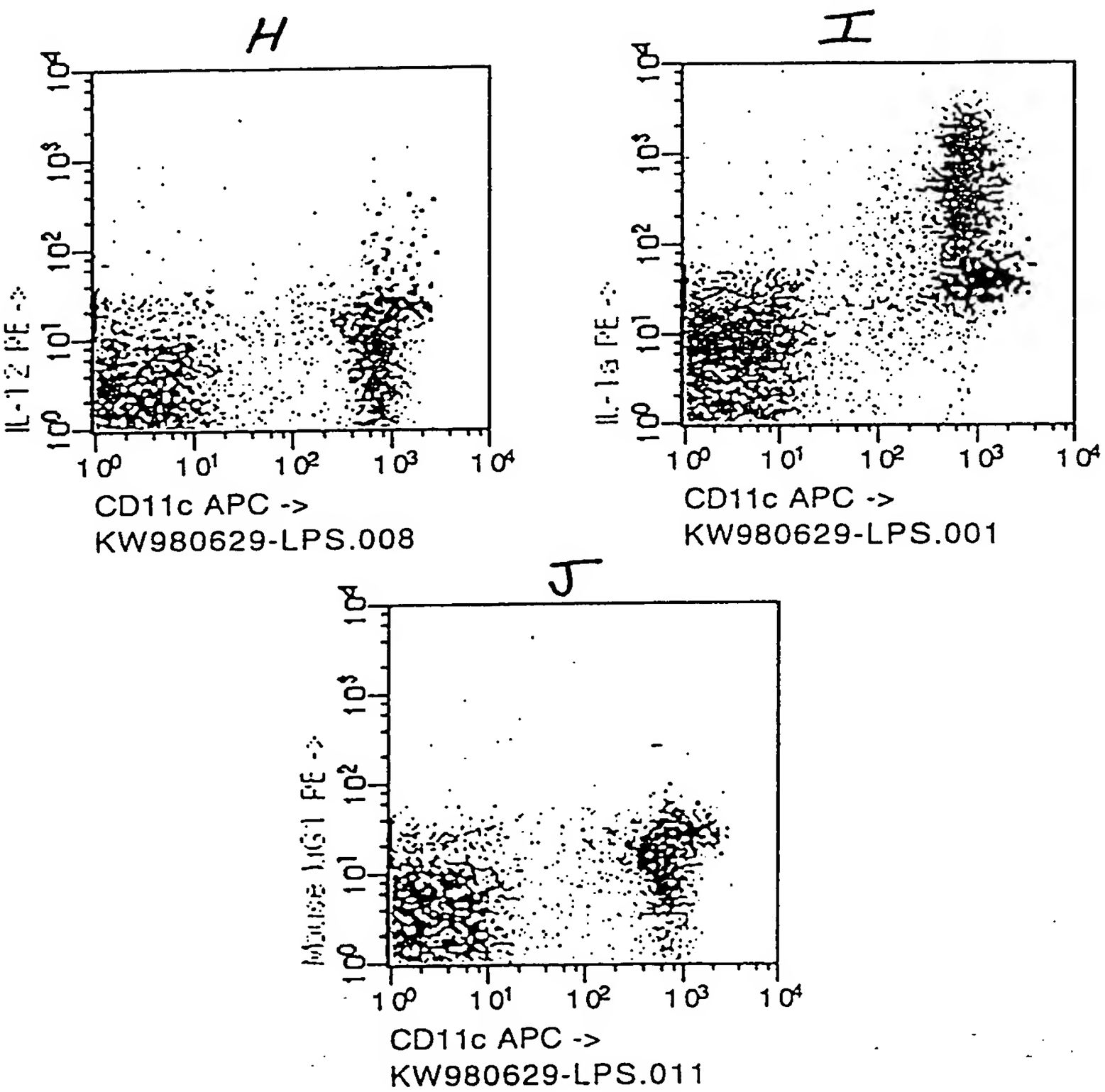


Fig 2 (CONTINUED)

Identification of CD11c+ and CD123+ (CD11c-) peripheral blood DCs

-PMA+I activated-

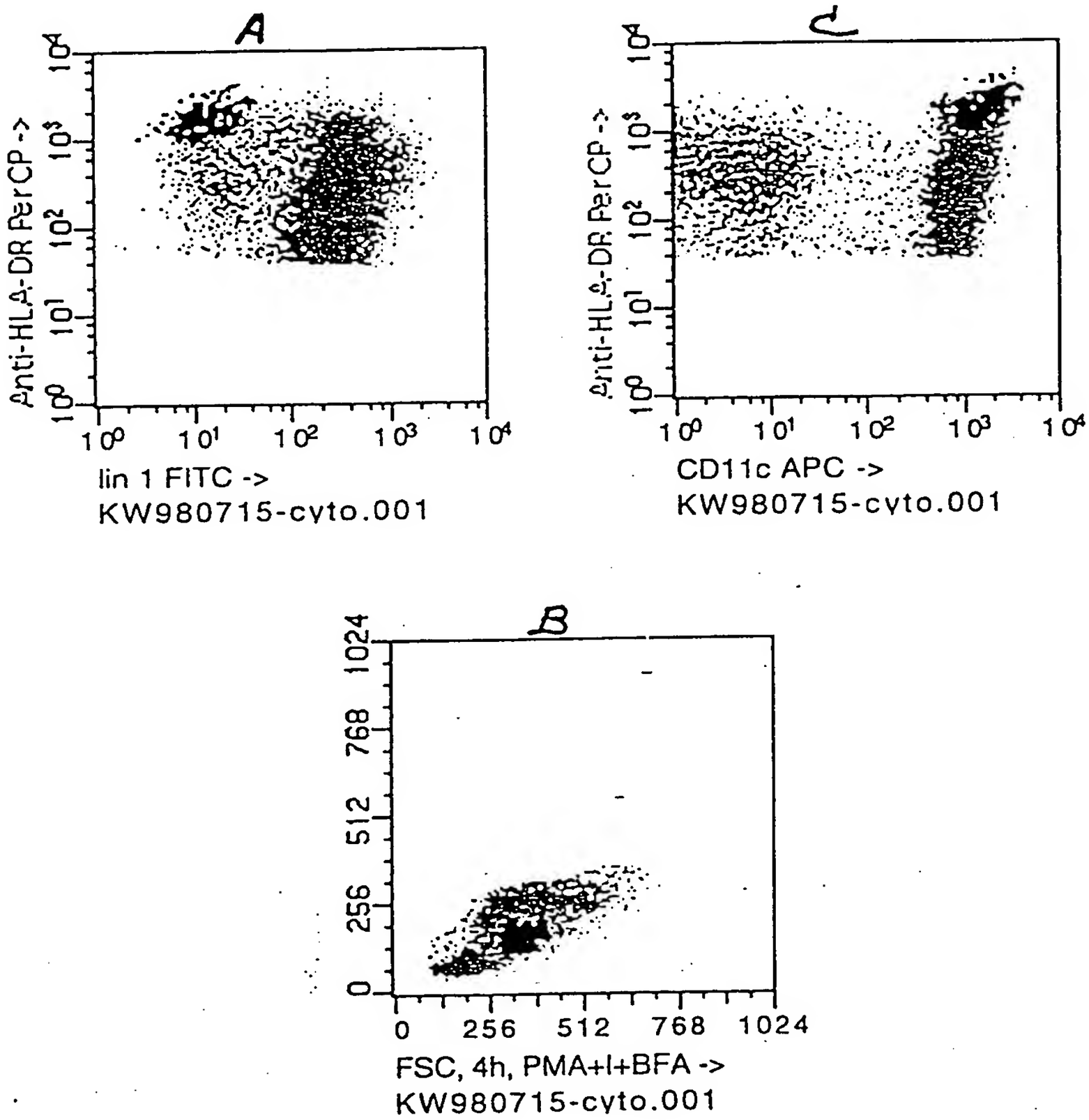


Fig 3

Detection of cytokines in CD11c+ and CD123+ (CD11c-) peripheral blood DCs

-PMA+I activated-

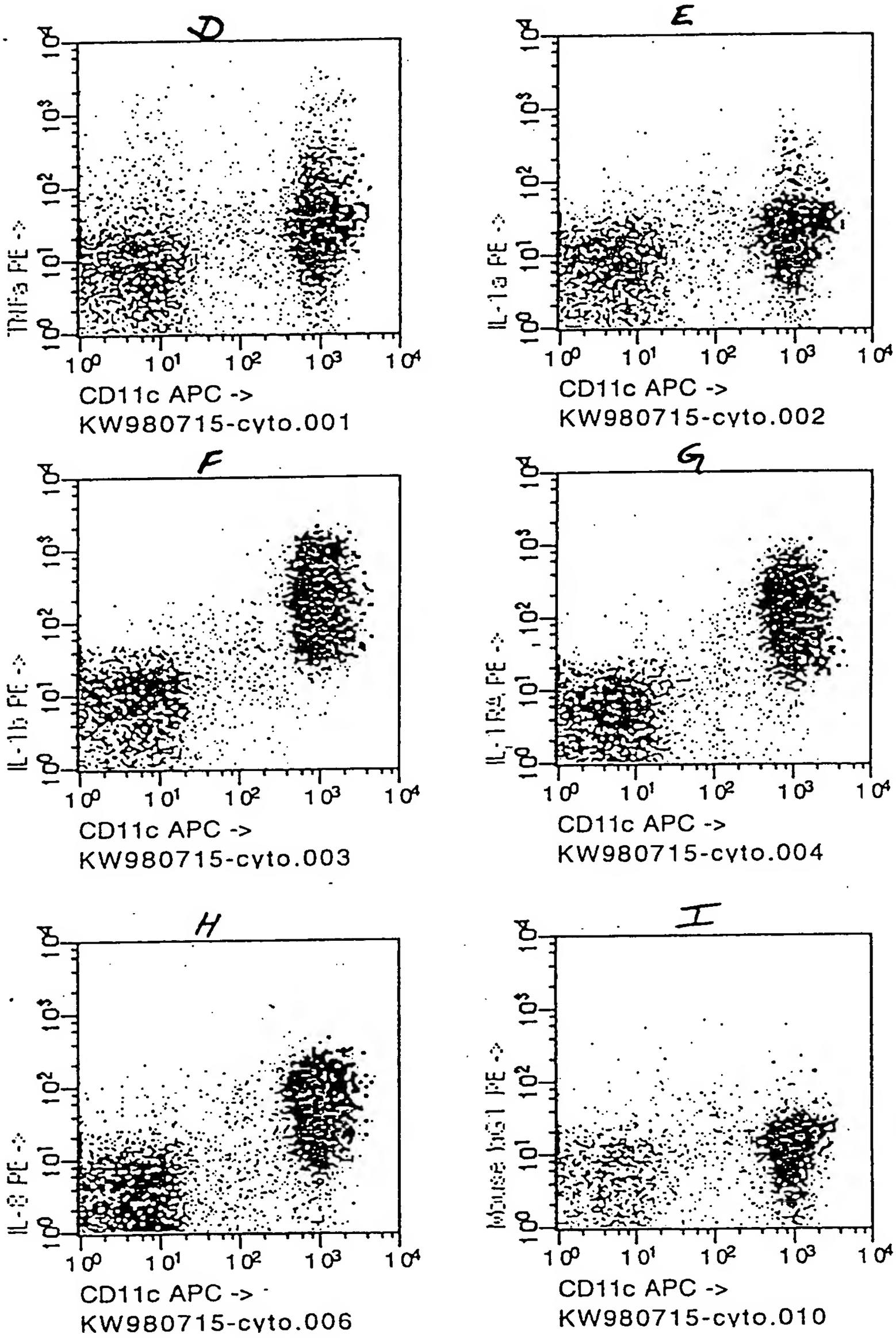


Fig 3 (CONTINUED)

Identification of CD11c+ and CD123+ (CD11c-) peripheral blood DCs

-BFA control-

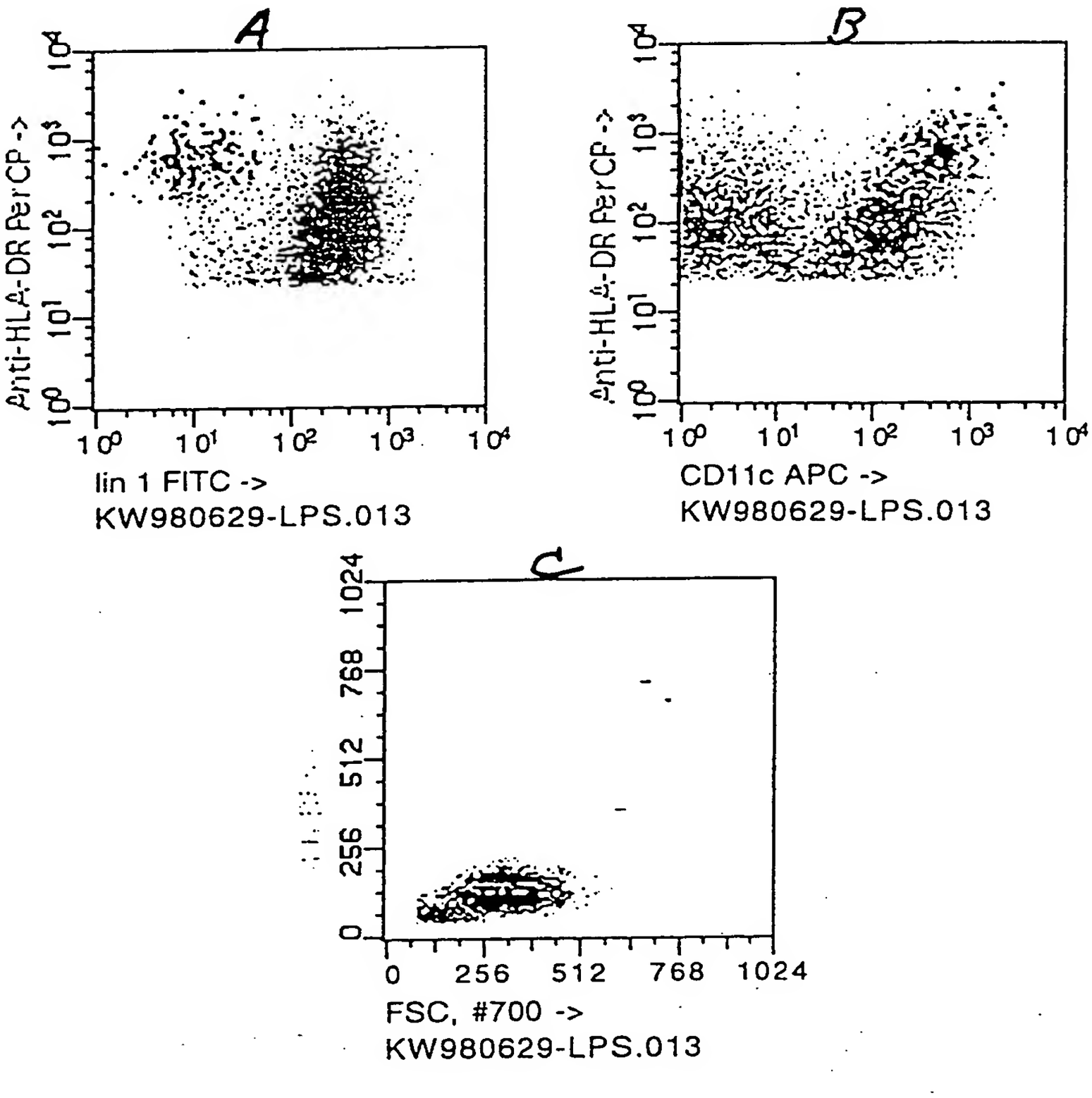
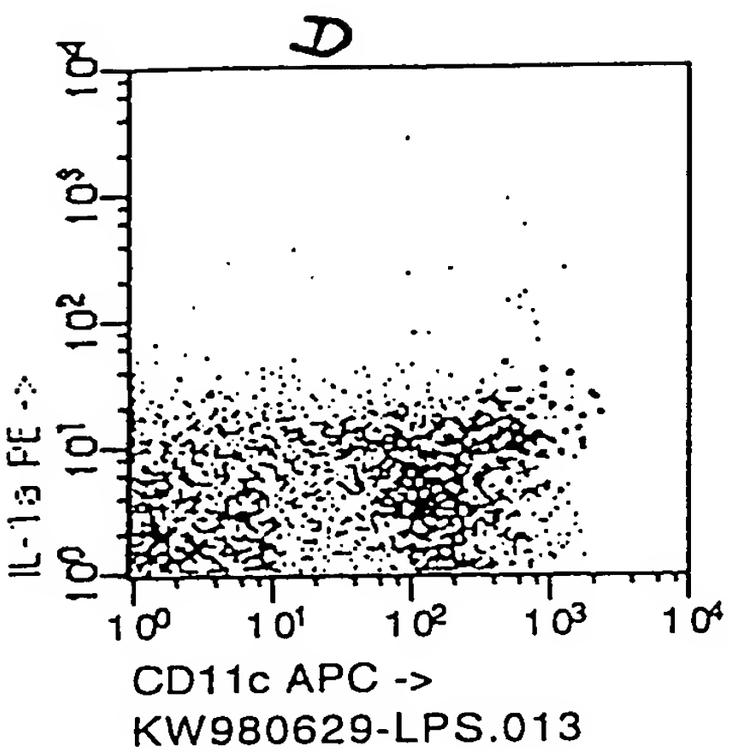
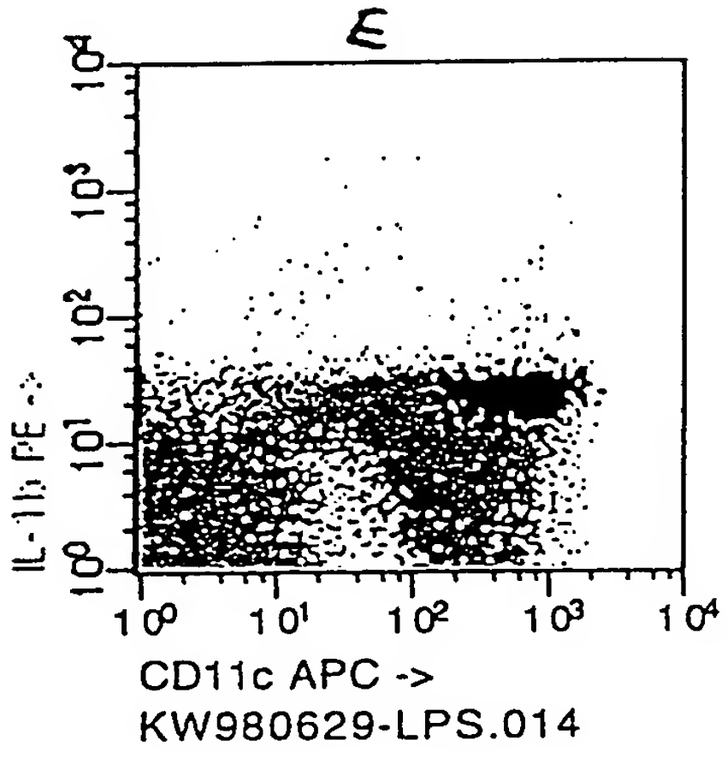


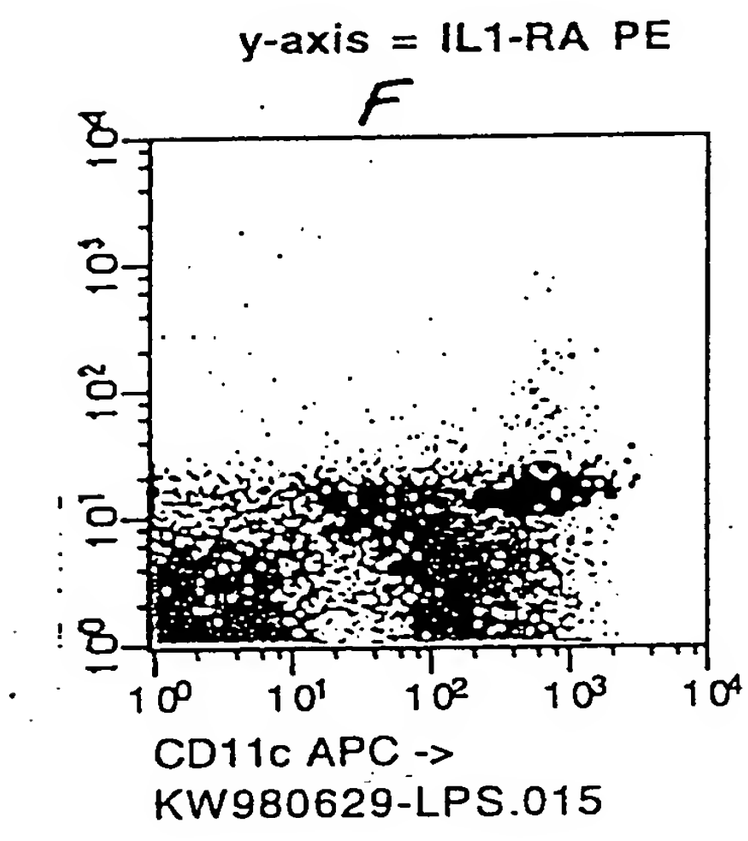
Fig 4

Detection of cytokines in CD11c+ and CD123+ (CD11c-) peripheral blood DCs

-BFA control-







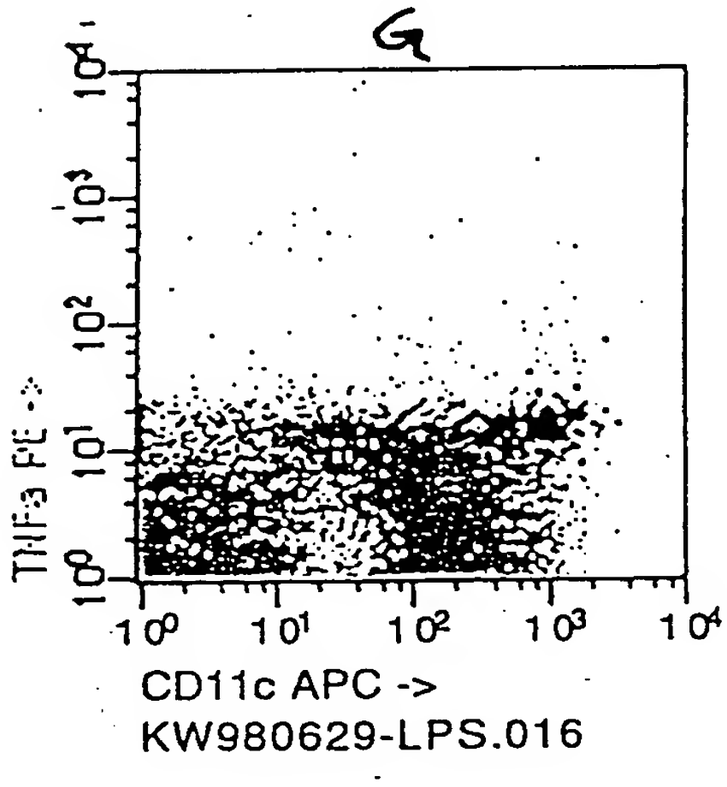
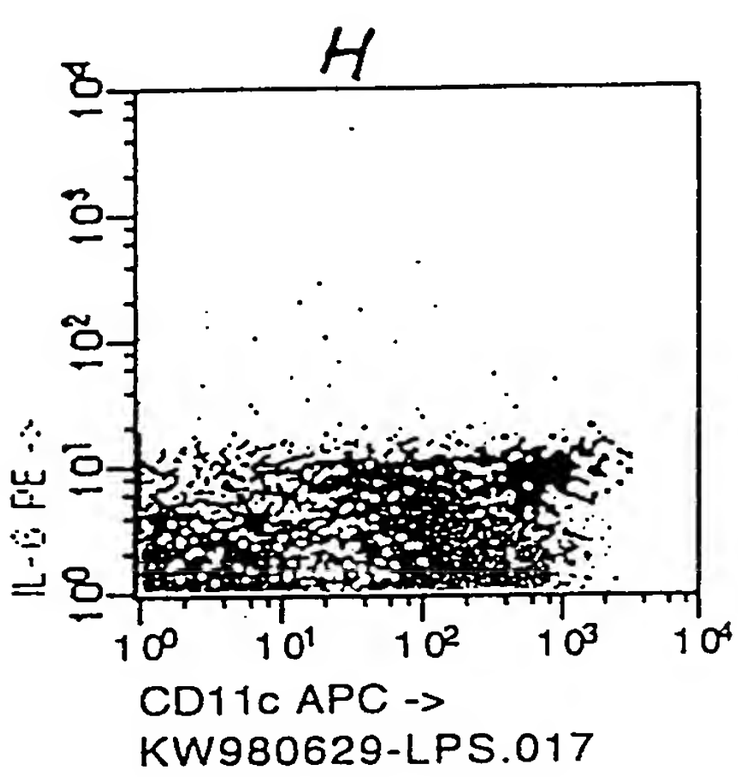
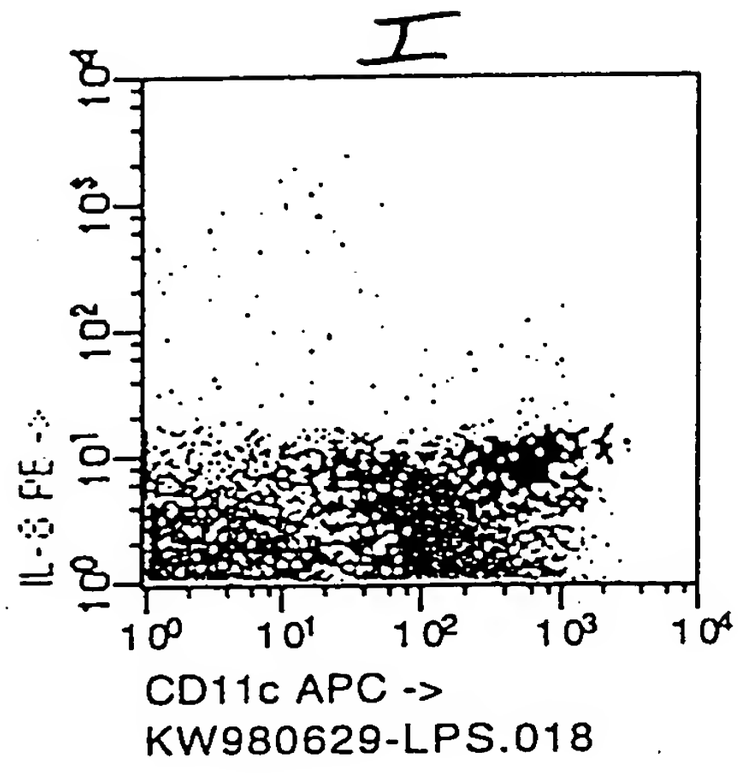


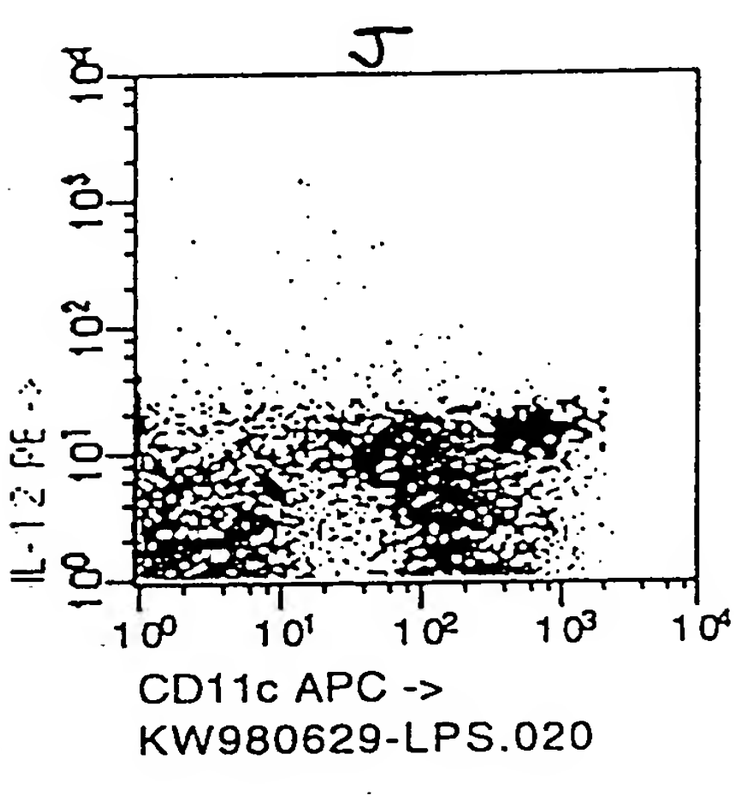
Fig 4 (CONTINUED)

Detection of cytokines in CD11c+ and CD123+ (CD11c-) peripheral blood DCs

-BFA control-







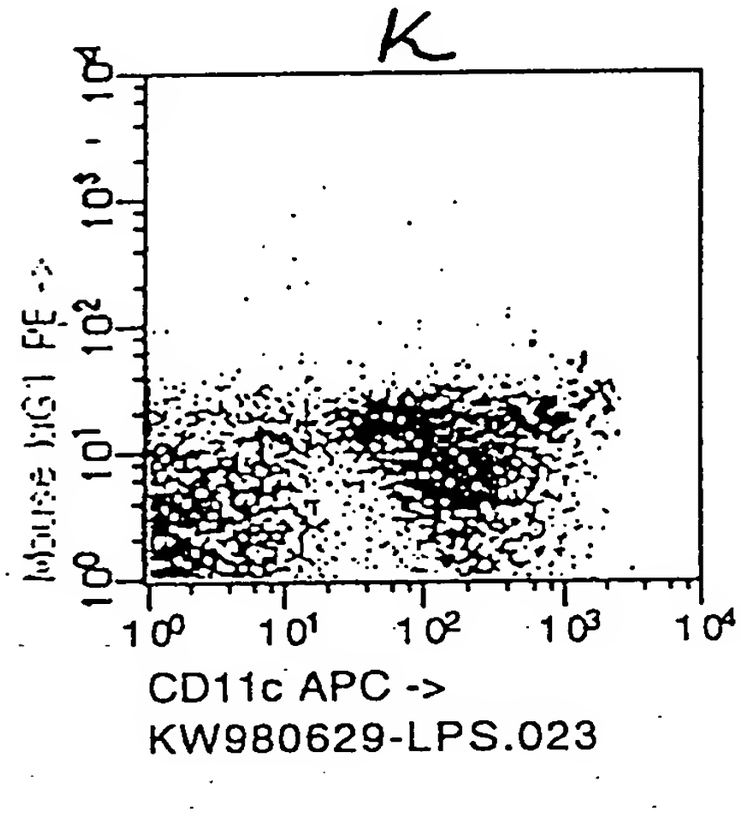


Fig 4 (CONTINUED)

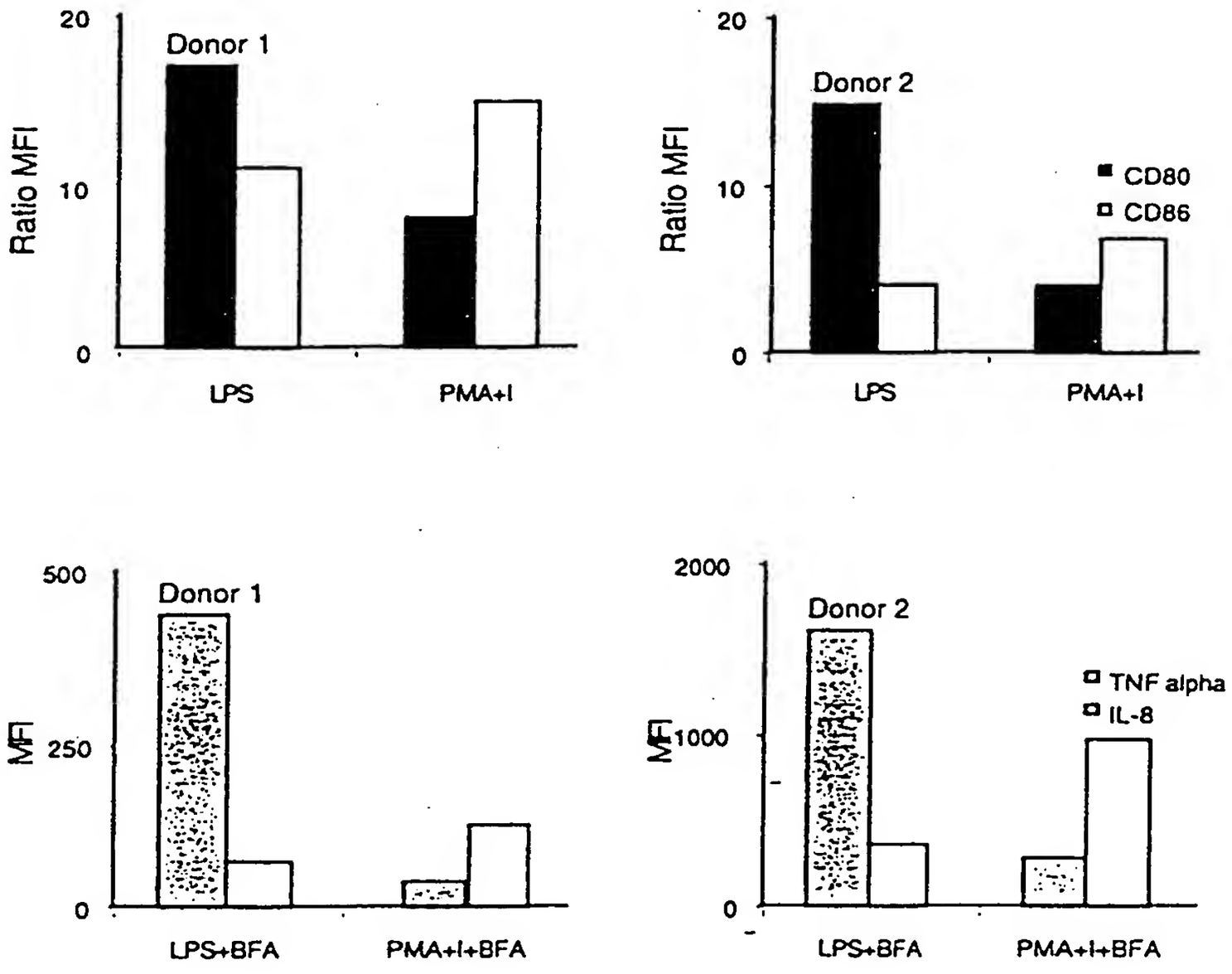
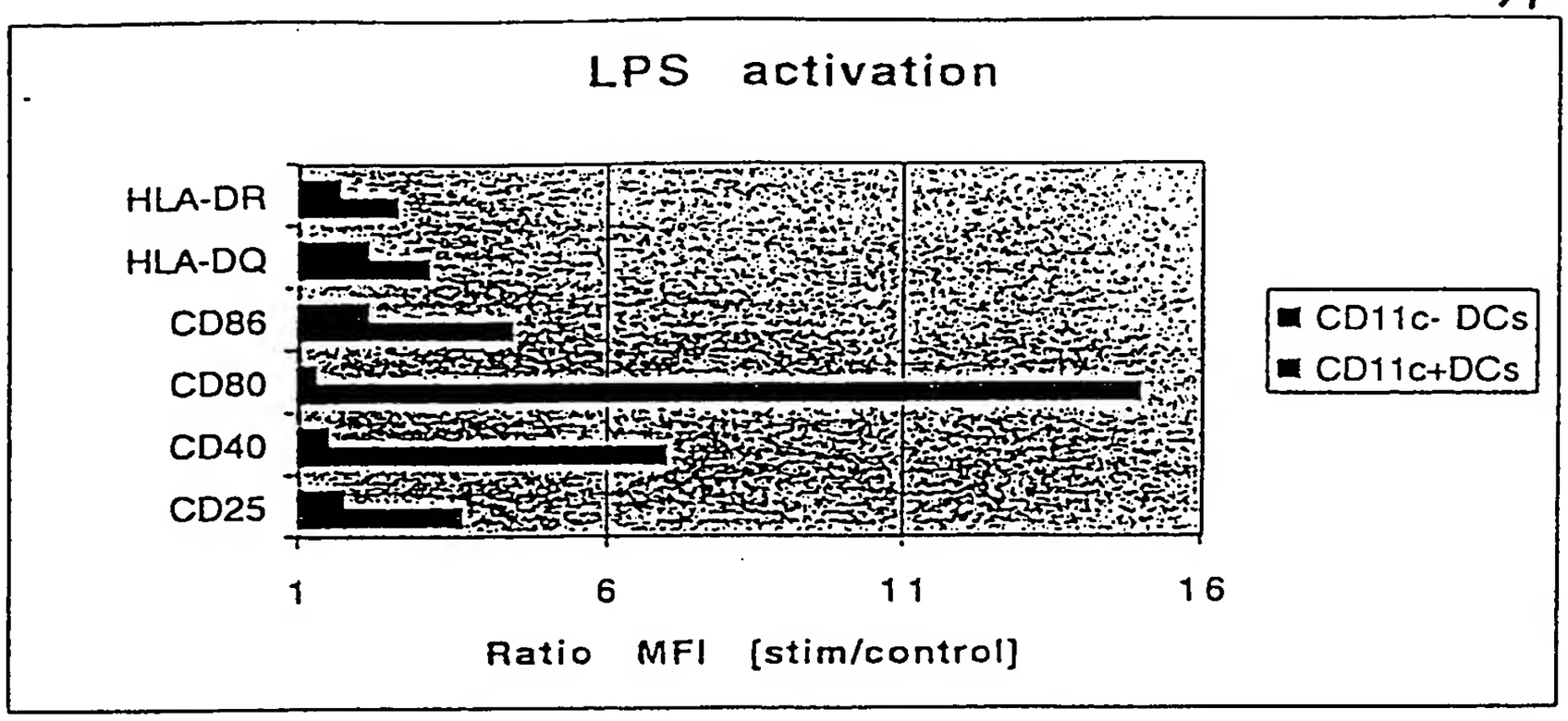

Donor 1
Donor 2
Ratio MFI
20
10
0
CD80
CD86
LPS
PMA+I
MFI
500
250
2000
1000
TNF alpha
IL-8
LPS+BFA
PMA+I+BFA

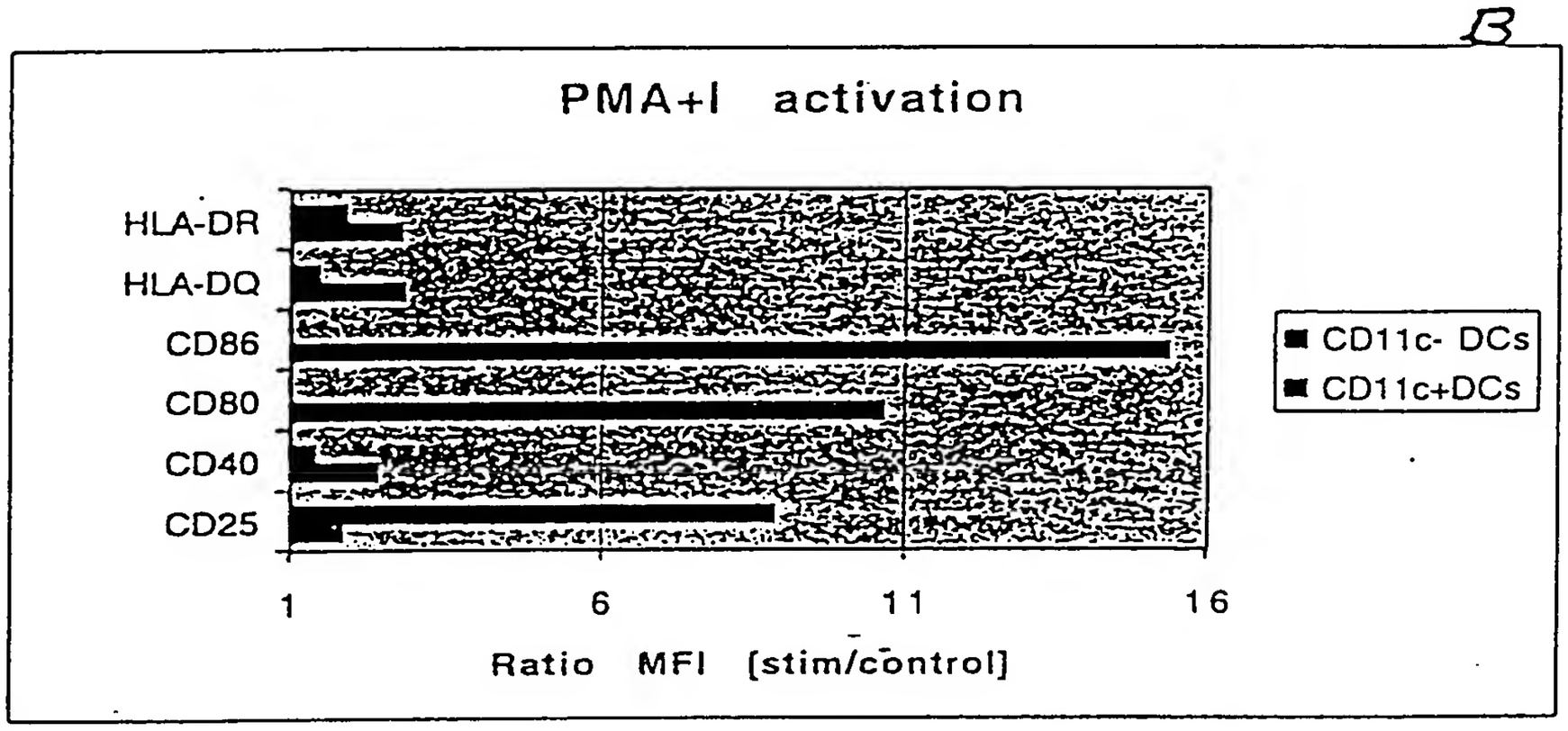
FIG. 5

Surface antigen expression in activated peripheral blood DCs

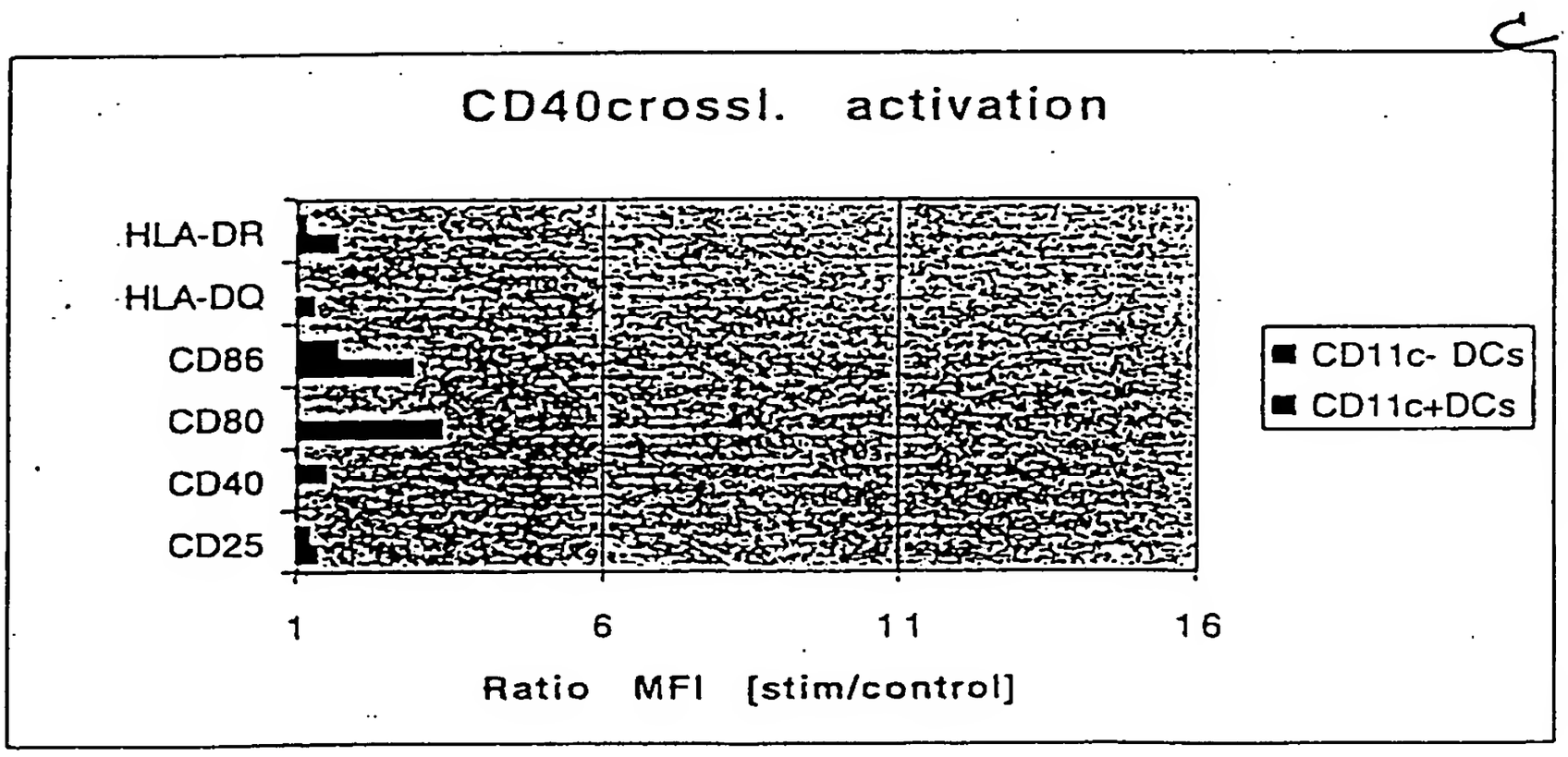
A



B



C

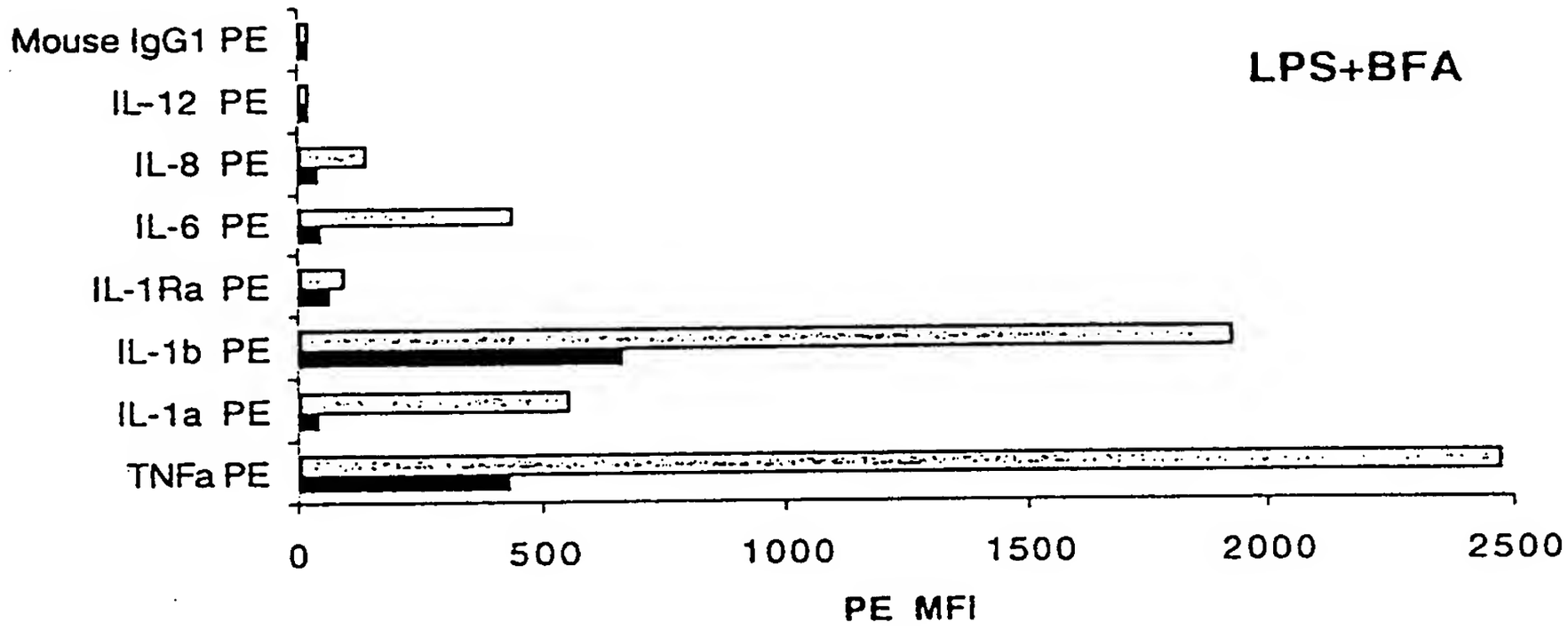


MFI = mean fluorescence intensity
stim = sample containing stimulus
control = sample kept at 37°C lacking stimulus

Fig 6

FIGURE 7

A


LPS+BFA
Mouse IgG1 PE
IL-12 PE
IL-8 PE
IL-6 PE
IL-1Ra PE
IL-1b PE
IL-1a PE
TNFa PE
0
500
1000
1500
2000
2500
PE MFI

B

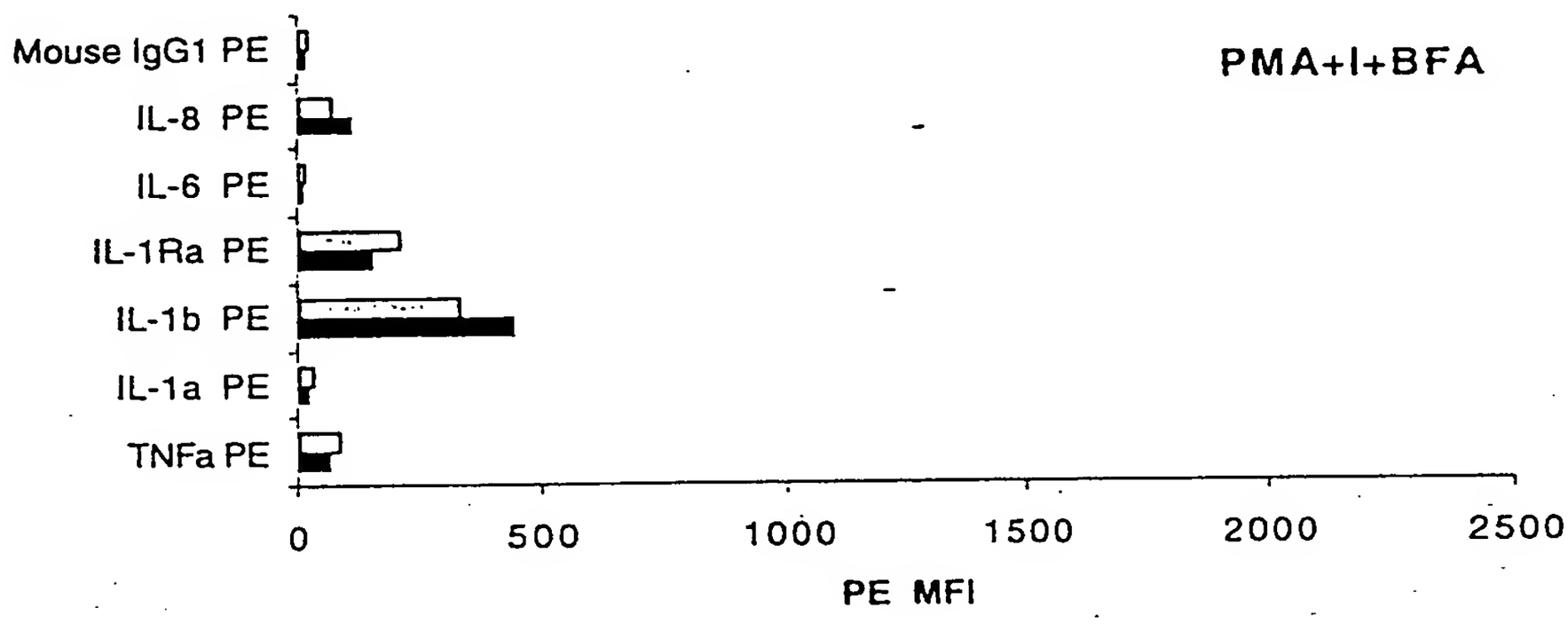
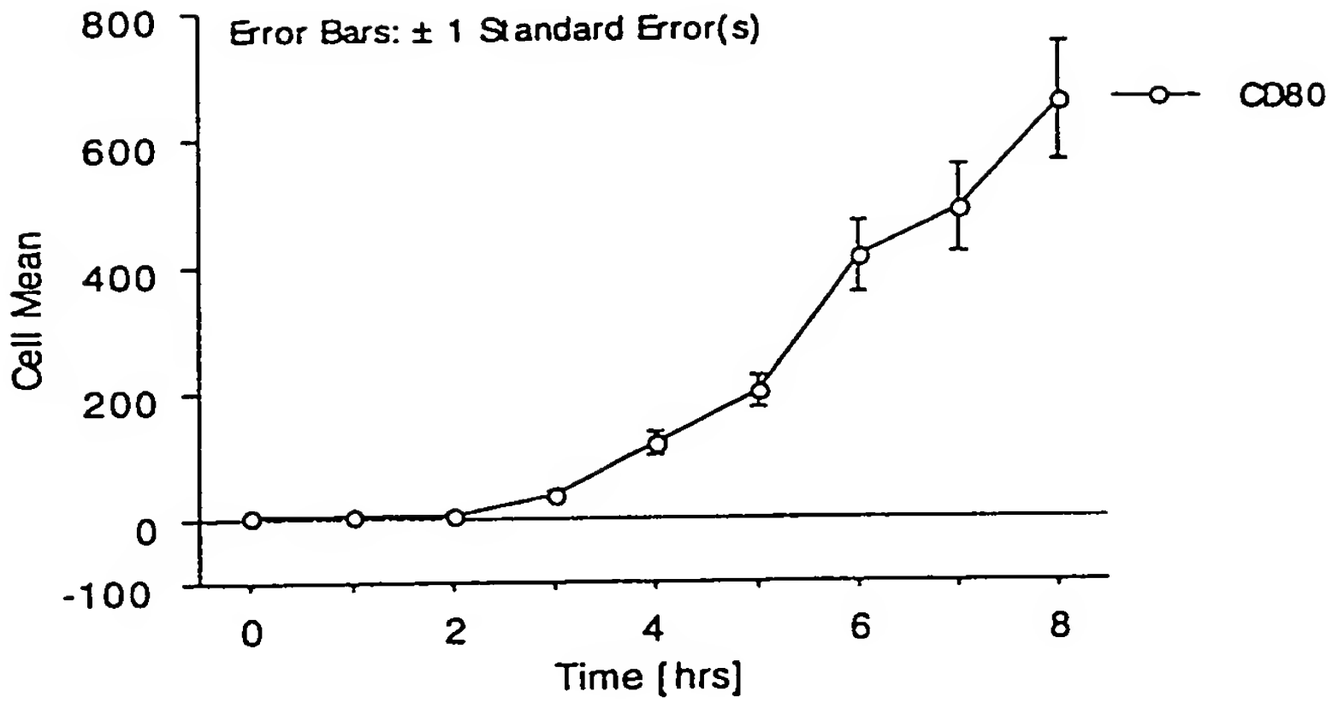
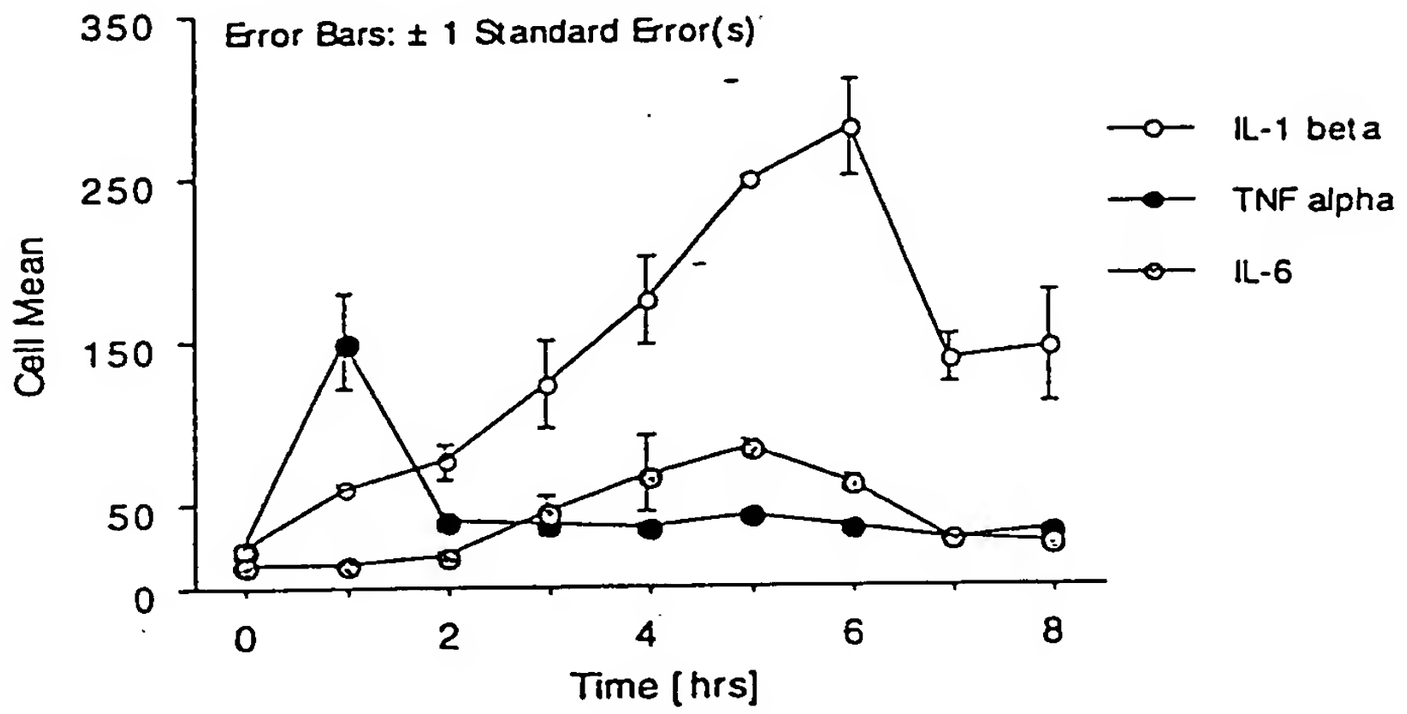

PMA+I+BFA
Mouse IgG1 PE
IL-8 PE
IL-6 PE
IL-1Ra PE
IL-1b PE
IL-1a PE
TNFa PE
0
500
1000
1500
2000
2500
PE MFI

FIGURE 8


Error Bars: ± 1 Standard Error(s)
800
600
400
200
0
-100
Cell Mean
0
2
4
6
8
Time [hrs]
CD80


Error Bars: ± 1 Standard Error(s)
350
250
150
50
0
Cell Mean
0
2
4
6
8
Time [hrs]
IL-1 beta
TNF alpha
IL-6